*Recibido el 31-05-2010*

# PAPER ELECTROPHORETIC TECHNIQUE IN THE STUDY OF BERYLLIUM(II) / COBALT(II) - PROLINE BINARY COMPLEXES IN SOLUTION

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## ABSTRACT

A method, involving the use of Paper Electrophoretic Technique (PET) is described for the study of binary complex systems in solution. This method is based on the movement of a spot of metal ion in an electric field at various pH of background electrolyte. A graph of pH versus mobility was used to obtain information on the binary complexes and to calculate its stability constants. The stability constant of Be(II) – proline and Co(II) – proline complexes were found to be (7.61 ± 0.11, 5.81 ± 0.07) and (4.97 ± 0.03, 3.69 ± 0.13) (logarithm stability constant values), respectively at ionic strength 0.1 Mol $L^{-1}$ and a temperature of 35° C.

**Key words**: Paper electrophoretic technique, overall mobility, beryllium(II) complexes, cobalt(II) complexes, stability constants.

## INTRODUCTION

Metal complexes play an important role in various biological systems, hence, the formation, stability and reactivity of these complexes have been an active field of research[1,2]. Beryllium and cobalt are toxic and essential metals respectively. The recommended amount of beryllium and cobalt in human diet are 0.01 mg/day and 0.005 - 1.8 mg/day, respectively. Cobalt is essential component of vitamin $B_{12}$-coenzymes. Inhaled beryllium compounds cause lung cancer. Beryllium inhibits phosphates enzymes and DNA replication. It is lethal at 1 ppm body weight. Deficiency of cobalt in human cause anemia while excess cause coronary failure[3]. It seems to very interesting to study the feasibility of controlling their level by complexation. Beryllium and cobalt have several significant applications in biological systems [4-6]. Proline or pryrolidine – 2 – carboxylic acid is an amino acid found in protein. Proline has several significant applications in biological systems[7-9].

The usual draw backs of PET like variation in the temperature during the electrophoresis, capillary flow on paper, electroosmosis and adsorption affecting the mobility of charged moieties, are quite well known[10]. The technique described here is almost free from these vitiating factors. The technique is very convenient in use and gives results in fair agreement with the accepted literature values.

Communications[11,12] from our laboratory described a new method for the study of metal complexes by paper electrophoretic technique. A search of literature indicated that no report is available on binary complexes of beryllium(II) and cobalt(II) with proline. In view of this, attempts were made to establish the optimum conditions for metal(II) – proline complex formation. In addition, present work describe a paper electrophoretic method for the determination of stability constants of these complexes.

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## EXPERIMENTAL

### Apparatus

A Systronic (Naroda, India) Model 604, electrophoresis was used. The apparatus consisted of a poly (vinyl chloride) PVC moulded double tank vessel. In our laboratory significant change in the instrument has been made. Two hollow rectangular iron plates each weighing one kg, and covered with thin polythene sheets have been used through which thermostated water circulated for controlling the temperature. The tanks are closed with a transparent PVC moulded lid. The whole assembly is tight to prevent moisture changes, which might upset the equilibria in the paper strip. This assembly design thus keeps to a minimum the disturbing effect of evaporation from the unwanted liquid flow in the paper strips. Each electrolyte tank contains a separate electrode chamber in which Pt-wire anode and cathode are placed, respectively. Applied voltage was from a stabilized source. The electrophoresis cell showing sandwiched paper strips and water supply is shown in figure 1.

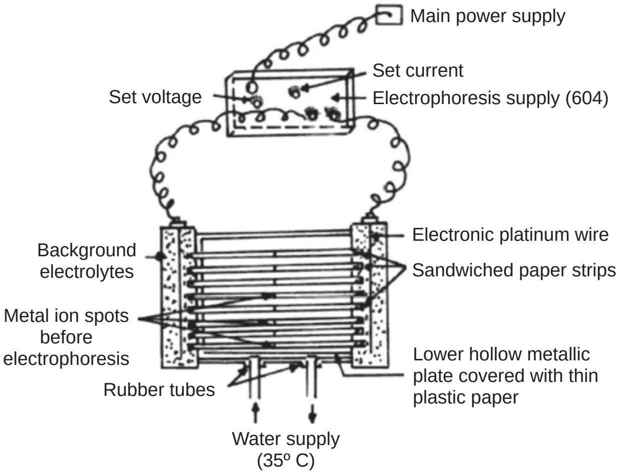


**Figura 1.** Electrophoresis cell showing sandwiched paper strips.

Whatman No. 1 filter papers for chromatography were used for the purpose of electrophoresis. Elico (Hyderabad, India,) Model $L_{1\text{-}0}$ pH meter using a glass and calomel electrodes assembly working on 220 V/50 Hz established a.c. mains, was used for the pH measurements. pH meter was calibrated with buffer solution of pH 7.0.

### Chemicals

### Preparation of metal solutions

Beryllium(II) and cobalt(II) metal perchlorate solutions were prepared by the precipitation of metal carbonates from a 0.1 Mol $L^{-1}$ solution of beryllium(II) and cobalt(II) nitrates with the solution of sodium carbonate (chemically pure grade, BDH, Poole, UK). The precipitates were washed with boiling water and treated with calculated amounts of 1 % perchloric acid. They were heated and filtered. The metal contents of the filtrates were determined and final concentration was kept 0.005 Mol $L^{-1}$ [13,14].

### Sodium hydroxide solution

Carbon dioxide free sodium hydroxide solution was prepared by dissolving 500 gms of sodium hydroxide in 500 mL of water in a flask. The flask was left overnight. The clear supernatant liquid was filtered rapidly using a high vacuum pump. A suitable volume of the filtrate was diluted and the concentration determined by titrating against a standard oxalic acid solution. A solution (2.0 Mol $L^{-1}$) was obtained by suitable dilution. The concentration of stock solution was checked from time to time.

### Detecting reagents for metal ions and glucose

Solution of 1-(2 – pyridylazo - 2 – naphthol (PAN) (E. Merck, Darmstadt, Germany), in ethanol was used for detecting the metal ions. 0.005 Mol $L^{-1}$ glucose (BDH, Analytical Reagent grade) solutions was prepared in water and used as an electro-osmotic indicator for the correction due to electro – osmosis. A saturated aqueous solution (0.9 mL) of silver nitrate was diluted with acetone to 20 mL. Glucose was detected by spraying with this silver nitrate solution and then with 2 % ethanolic sodium hydroxide, when a black spot was formed. Paper strips showing position of metal ion spots after electrophoresis is shown in figure 2.

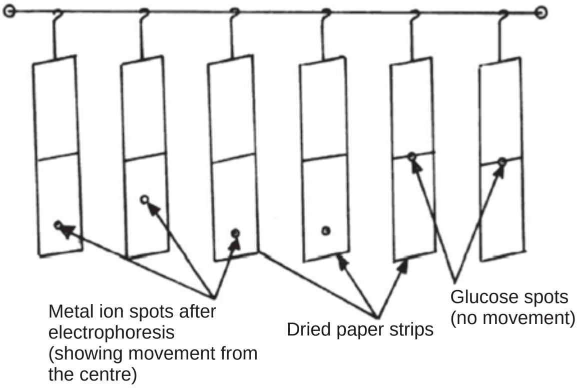


**Figura 2.** Paper strips showing position of metal ion spots after electrophoresis

### Background electrolyte

The background electrolytes used in the study of binary complexes were 0.1 Mol $L^{-1}$ perchloric acid and 0.01 Mol $L^{-1}$ proline. The system was maintained at various pH by the addition of sodium hydroxide. Stock solution of 5.0 Mol $L^{-1}$ perchloric acid (SDS, analytical reagent grade), 2.0 Mol $L^{-1}$ sodium hydroxide (analytical reagent grade) and 0.5 Mol $L^{-1}$ proline were prepared. Each solution was standardized using the appropriate method.

### Procedure

Whatman No. 1 filter paper for chromatography was used for the purpose of electrophoresis. For recording observation of particular metal ion, two strips were spotted with the metal ion solution along with additional two spotted with glucose using 1.0 µL pipette and then mounted on the insulated plate. Each of the two electrolyte vessel was filled with 150 mL of background electrolyte containing 0.1 Mol $L^{-1}$ perchloric acid and 0.01 Mol $L^{-1}$ proline. The paper became moistened with the background electrolyte solutions due to diffusion. The second insulated plate was placed on paper strips and then thermostated water (35° C) was circulated in the plates to keep the temperature constant. The lid was then placed on the instrument to make it airtight. It was left for 10 minutes to insure wetting of strips.

Subsequently a direct 200 Volts potential was applied between the electrodes. Electrophoresis was carried for 60 minutes after which these strips were removed from the tank and dried. The metal ion and glucose spots were detected by specific reagents. The leading and tailing edge were measured from the marked centre point and the mean were taken. The distance moved by glucose was subtracted (in case of migration toward anode) to obtain correct path length. Migration towards anode and cathode were designated by negative and positive signs respectively.

Electrophoretic observations on metal ions were recorded at various pH values of the background electrolyte obtained by adding sodium hydroxide solution. The ionic strength being maintained at 0.1 Mol $L^{-1}$. The observed mobility of migrant was calculated by using the formula.

$$U = \frac{d}{x \cdot t}$$

after applying the correction factor the observed mobility is given as

$$U = \frac{d \pm d_G}{x \cdot t}$$

where U = mobility of metal ion / complex ion; d = mean of duplicate distance travelled by metal ion/complex ion; $d_G$ = mean of duplicate distance travelled by glucose spot; x = field strength; t = time for electrophoresis. The mobility of metal / complex ion spots on the paper strips were thus calculated and are reported with different pH values (figure 3).

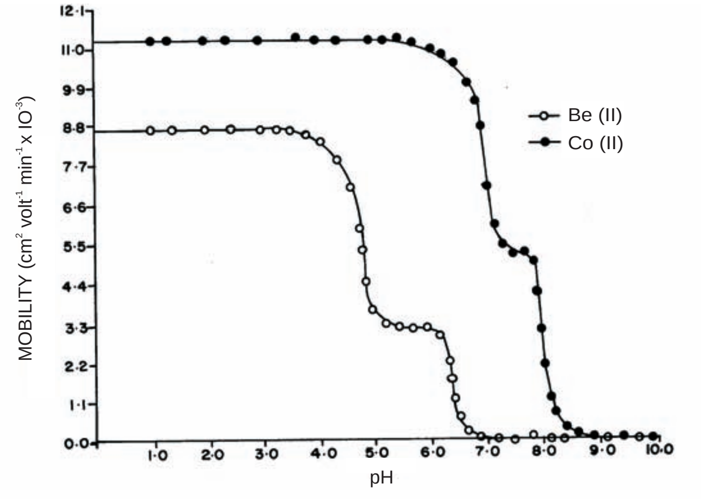


**Figure 3.** Mobility curve for the metal(II) - proline systems. —○— = Be(II) - proline —●— = Co(II) - proline. Background electrolytes: 0.1 M perchloric acid and 0.01 M proline. pH's were maintained by addition of sodium hydroxide. The paper strips were spotted with 0.1 µL of sample solutions and glucose (for making osmotic corrections).

The protonotation constants of pure proline were determined by the same paper electrophoresis technique. The two paper strips were spotted with pure proline along with two with glucose using 0.1 Mol $L^{-1}$ perchloric acid only in a background electrolyte. The electrophoresis was carried for 60 minutes as for metal ions. The electrophoretic speed was calculated. The speed of the metal ion/proline spots are reported with pH values. The individual speeds of the duplicate spots were found to be fairly equal.

## RESULTS

Literature reveals that an ionic species of amino acids are the sole coordinating species in complex formation with metal ions[15, 16]. Hence a metal ion spot on the paper strip show a variation in composition of different ionic species of the amino acids in background electrolyte. So the mobility of metal ion spot would depend upon the pH of the background electrolyte.

The electrophoretic mobility of a metal spot against a pH gives a curve with the number of plateau are shown in figure 3. Every plateau indicates the formation of certain complex species. A plateau is obviously an indication of a pH range, where speed is practically constant. The first plateau corresponds to a region, where metal ions are uncomplexed. In this low pH region protonated ionic species of proline is maximum. The second plateau in each case with positive mobility indicates a 1:1, complexes of cationic nature. In this high pH region proline present in the anionic form $[L^-]$.

One species of proline anion combine with each divalent metal ion to form $[BeL]^+$ and $[CoL]^+$ cationic complexes, respectively. At higher pH ranges third plateau was observed in each case at the zero region of mobility curve, which indicates formation of 1:2 metal complexes of neutral nature. The two anionic species of proline combined with metal ion to form $[BeL_2]$ and $[CoL_2]$ neutral complexes, respectively. Since there is no fourth plateau, no further interaction with proline anion possible.

The 1:1 and 1:2 metal complexes are formed at pH range 1.0 – 8.5. Chemical literature also assigns a prominent chelating property to the zwitterions[17,18]. The complexation of $Be^{2+}$ and $Co^{2+}$ with proline anion $[L^-]$ may be represented as

$$M^{2+} + L^- \overset{K_1}{\leftrightarrows} ML^+ \qquad (1)$$

$$ML^+ + L^- \overset{K_2}{\leftrightarrows} ML_2 \qquad (2)$$

where $M^{2+}$ is $Be^{2+}$ and $Co^{2+}$ metal ions; $[L^-]$ is the proline anion; $K_1$ and $K_2$ are the first and second stability constants, respectively.

The metal spot on the paper is thus a combination of uncomplexed metal ions, 1:1 and 1:2 metal complexes. The spot is moving under the influence of electric field and the overall mobility is given by equation of Jokl[19].

$$U = \frac{\Sigma u_{xp} \, . \, \beta_{xp} \, [HpL]^{x}}{\Sigma \, \beta_{xp} \, [HpL]^{2}} \qquad (3)$$

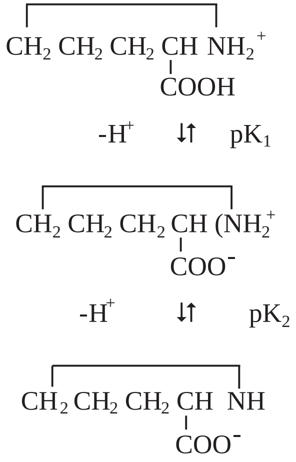
wherein $[HpL]^x$ is the concentration of general complex species; $\beta_{xp}$ is the overall mobility constant of the complex; $u_{xp}$ is the speed of the general complex $[M(HpL)^x]$ present in the conglomeration.

On taking into consideration different equilibria, the above equation is transformed into the following form:

$$U = \frac{u_0 + u_1 K_1 [L^-] + u_2 K_1 K_2 [L^-]^2}{1 + K_1 [L^-] + K_1 K_2 [L^-]^2} \qquad (4)$$

wherein $u_0$, $u_1$ and $u_2$ are mobilities of uncomplexed metal ion, 1:1 metal complex and 1:2 metal complex, respectively.

The protonation constant of pure proline ($pK_1 = 1.90$; $pK_2 = 10.03$) were obtained by same paper electrophoretic technique. The mode of deprotonation of pure proline can be represented as:



With the help of protonation constants of pure proline, the concentration of the ligating proline $[L^-]$ is calculated.

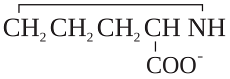
$$[L^-] = \frac{[L_T]}{1 + [H] / pK_2 + [H]^2 / pK_1 \, . \, pK_2} \qquad (5)$$

wherein $[L_T]$ is total concentration of proline (0.01 Mol $L^{-1}$); $pK_1$ and $pK_2$ are first and second protonation constants of pure praline, respectively.
For calculating first stability constant, $K_1$, the region between first and second plateau is pertinent. The overall mobility will be equal to the arithmetic mean of the mobility of uncomplexed metal ion, $u_0$, and that of first complex, $u_1$ at a pH where $K_1 = 1/[L^-]$.
The stability constant $K_2$, of second complex can be calculated by taking into consideration the region between second and third plateau of the mobility curve. The calculated values of first and second stability constants are given in table 1.

**Table 1**. Stability constants of beryllium(II) and cobalt(II) complexes with proline.

| **Metal ions** | **Complexes** | **Stability constants** | **Logarithm stability constant values** |
|---|---|---|---|
| Beryllium(II) | $Be\{CH_2\,CH_2\,CH_2\,CH\,NH_2\,COO\}^+$ | $K_1$ | 7.61 ± 0.11 |
| | $Be\{CH_2\,CH_2\,CH_2\,CH\,NH\,COO\}_2$ | $K_2$ | 5.81 ± 0.07 |
| Cobalt(II) | $Co\{CH_2\,CH_2\,CH_2\,CH\,NH_2\,COO\}^+$ | $K_1$ | 4.97 ± 0.03 |
| | $Co\,\{CH_2\,CH_2\,CH_2\,CH\,NH\,COO\}_2$ | $K_2$ | 3.69 ± 0.13 |

Ionic strength = 0.1 Mol $L^{-1}$; temperature = 35° C; M = metal cations,($Be^{2+}$ and $Co^{2+}$); L = Ligand (proline); proline anion =



$CH_2\,CH_2\,CH_2\,\underset{COO^-}{CH}\,NH$

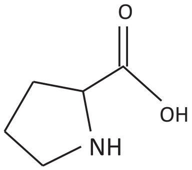
## DISCUSSION

Comparison of log stability constants values for both metal ion complexes follow the order:

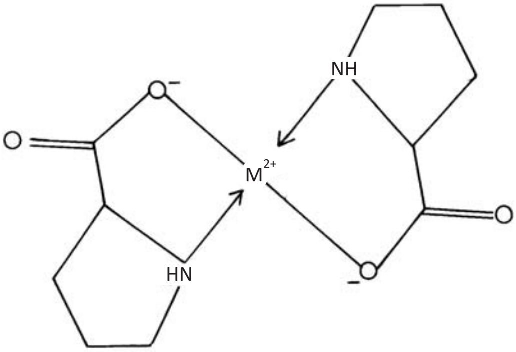
$$\log K_1 > \log K_2$$

The values of second stability constants in each complex found to be lower in comparison to the first stability constant values. It is therefore inferred that the coordinating tendency of a ligand decreases with higher state of aggregation[20]. High stability constant values of beryllium(II) – proline complexes indicate strong bonding between beryllium(II) cation and proline anion. While low stability constant value between cobalt(II) – proline complexes indicate weak bonding between cobalt(II) cation and proline anion.

The higher stabilities of beryllium(II) complexes may be ascribed to be its greater affinity for the oxygen donor ligands. The precision of the method is limited to that of paper electrophoresis, and uncertainty in the result is ± 5 %. Hence, it can not replace the most reliable methods, even though it is new approach deserving further development. The stability constants of metal complexes, can be very easily calculated by this technique; therefore the present method is advantageous over other methods (viz., polarography, potentiometry, solubility, etc.) reported in chemical literature. The molecular structure of proline is as follows:



To examine the possibility of hydrolysis of beryllium(II) at higher pH, experiments have been performed at two concentrations of the ligand : 0.01 Mol $L^{-1}$ and 0.001 Mol $L^{-1}$. The mobility curves show that the plateaus at lower ligand concentration are shifted towards higher pH range, but the calculated stability constants are found to be the same in the two cases. Thus the constant obtained is independent of the pH indicating that hydrolysis of beryllium(II) can be ignored here. The proposed structure for the $ML_2$ complexes may be given as:



## CONCLUDING REMARKS

The following conclusions can be drawn from the present study

- Beryllium(II) and cobalt(II) are significant for biological systems as such they are toxic, the proline may be used to reduce the level of these metal ions in the biological systems.
- Beryllium(II) – proline complexes have high stability constant values in comparison to cobalt(II) – proline complexes.
- The present paper electrophoretic technique is very helpful in finding that complex system is formed or not; if formed, its stability constants can also be determined.
- The $ML_2$ complexes have low stability constant values less stable in comparison to ML complexes.
- Biologically important beryllium(II) and cobalt(II) complexes with proline can be prepared on large scale at particular pH of the background electrolyte solutions.

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